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User Manual for LLNL's 24 Chamber Instrument

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User Manual for LLNL's 24 Chamber Instrument

(a.k.a. PolyHanaa)

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3 / 29 / 01

Table of Contents

PolyHanaa Introduction

The 24 chamber instrument, called the PolyHanaa, is designed to perform rapid, real-time detection of biological agents using the Polymerase Chain Reaction (PCR) process. Liquid samples are pipetted into small, disposable polypropylene inserts which are placed into each of the 24 thermal cycling chambers.

Section 1. Igor Pro Overview

The user interface for the PolyHanaa is written using the application Igor Pro (WaveMetrics, Inc., Lake Oswego, OR).

Input methods in Igor

Interactions take place via buttons on permanent panels or via pull-down menus. The main menu bar contains a combination of Igor's built-in menus and LLNL designed menus. The LLNL menus have a 'p.' prefix and appear on the right side of the menu bar. In addition to the menu bar, users can execute specific commands on the Igor Command Line, which is at the bottom of the Command Window (Figure 1). It is accessible using the key combination Command - J. The command line is a normal input mode for Igor but isn't essential to operate the 24 chamber unit. A history of previously executed commands is retained in the upper portion of the Command Window above the Command Line.

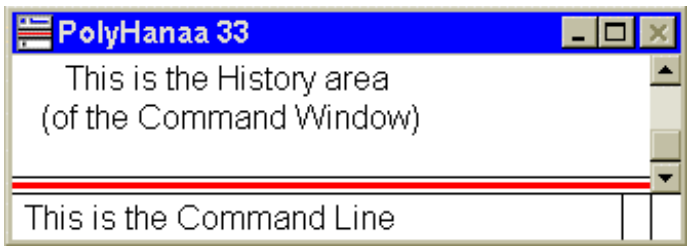

Figure 1. The Igor Pro Command Window

Igor terminology

In order to take advantage of the varied features associated with Igor Pro and to better understand how the PolyHanaa is designed, a quick introduction to the terminology of Igor is useful.

A 'wave' is a one dimensional array of values. For example, a graph plots a signal 'wave' vs. a time 'wave'.

An 'experiment' is Igor's name for a main file. It is a collection of Igor objects, including waves, variables (numeric or string), graphs, tables, control panels and procedures. An Experiment appears as a single file on the PC.

A 'data folder' is a sub-collection of waves and variables within the Experiment. On the 24 chamber instrument, data folders are mostly used to store data from past PCR runs.

Closing Igor windows

When closing Igor windows (graphs, tables, notebooks, panels, etc.), the options presented are sometimes confusing. Primarily, this is because Igor uses the terms 'Kill' and 'No Save' in a way that typically means 'Close' (as opposed to 'Delete'). This helps explain options such as 'Save and then Kill'. An effort has been made to avoid confusion by designing the system such that the user can't corrupt LLNL generated software routines. However, such protection is not absolute and the user needs to be aware of potential pitfalls. This information will also help to deal with user generated graphs, tables and 'notebooks' (text files).

Values entered in tables are essentially saved as soon as they are entered. The options offered upon closing relate to the table's size, format and location.

To make things simpler, all LLNL generated tables are designed such that the close box bypasses the dialog window. However, if such tables are left open upon closing the Experiment, when the Experiment is reopened, the close box will show the dialog. Selecting 'No Save' will preserve the close box bypass feature.

Important Note: With all LLNL generated windows, ALWAYS select 'No Save' (NEVER select 'Replace').

Using Igor Help, Getting Help

The Igor Help system is extensive and easy to use. Simply select the Help Browser from the Windows menu and select the topic of interest.

If questions or problems can't be resolved with this manual, please call the number listed at the top of this document.

Section 2. Operational Overview

Operational Essentials

To avoid problems such as corruption of software, damage to hardware and misleading assays, ALWAYS follow these guidelines:

Management of files and windows;
- Make regular backups of the Experiment.
- Save the Experiment after each calibration.

Handling inserts and samples;
- Be very careful when inserting inserts into chambers. Insert them slowly, keep them vertical and never force them.
- Always use a full sample volume of 25 µl.
- Adjust integration time to avoid low signal levels.
- Use the lower (Channel B) assay for FAM based probes.

Maintaining the Experiment

PCR run data is automatically saved to external, desktop folders. These folders should be considered as a backup/archive system. Any changes made to settings during operation of the PolyHanaa, including automatically generated calibration settings and the automatically generated data folders within the Experiment, are not automatically saved at the system level. When new values are entered (and apparently saved), they are only saved locally as current values; if the system was shut down without saving the Experiment, those settings would be lost. For this reason, it is advisable to Save the Experiment on a regular basis and to always save it before shutting down the system.

There are several potentially negative implications to running an Experiment for an extended time even if it is frequently saved (as opposed to Saved As…). First, information will be lost from the History window since it has a limited size. While this information is not essential, a user may find it useful as a log that details activities. Since a lengthy history text slows operation of the processor, Igor has a Preference setting to allow for an automatic limit on the number of lines retained.

It is also advisable to make backups of the experiment on a regular, perhaps daily basis using the 'Save Experiment As…' option.

The size of the experiment (kilobytes on disk) tends to increase during use because of the History text and because the number of data folders increases every time a PCR run is finished. While neither of these factors significantly slow down system operation, they do take up disc space, especially if they are repeatedly duplicated as backup files. Therefore, it is advisable to save the History (if desired) separately to a notebook file and to create archive experiments which only contain data folders (that is, they can't run PCR). Once the History text and data folders are backed up, they can be deleted (see below), making the Experiment much smaller in memory.

History Text

The length of the History text can be automatically limited by using the menu path: Misc > Miscellaneous Settings… > Category = Command Settings > Limit Command History Text > (enter the number of lines). The PolyHanaa is shipped with the number of lines limited to 500. This means that History Text beyond 500 lines will be lost. A good way to save History text is to copy it to an Igor Notebook file which can be saved on the desktop. Notebook files are handy text files that can be generated by the menu path: Windows > New > Notebook… > ('plain text' is OK). The sequence of events would be as follows; prior to closing the Experiment, open the Notebook file (File > Open File > Notebook…), scroll down to the bottom, click anywhere in the History window, type Command + A (Select All), then copy and paste into the notebook. Save the notebook (File > Save Notebook), then delete the History by selecting all then pressing delete. Now, when the Experiment is Saved (or Saved As…), it will have a clean slate in the History window.

Saving Data Folders outside the main Experiment

The simplest way to save data folders is to Save the Experiment As… (an archive name). The intention is not to use the archive Experiment for running PCR, but only for using the Analysis menu. However, this method is inefficient in that it also saves all the other overhead information that defines the Experiment. Special archive experiments have been provided which are streamlined to only support analysis (not PCR). Data folders can easily be moved from the main experiment to such an archive experiment using the 'Data Browser' (Data > Data Browser) 'Browse Expt…' option. The procedure would be as follows:

Save the current experiment
Open the archive experiment (DF Archive 1, DF Archive 2, etc.)
Open the Data Browser
Select 'Browse Expt…'
Choose the current experiment (its data folders will appear)
Drag data folders from the current experiment into the archive
Select 'Done Browsing'

The next time you open the current experiment, you can open the data browser, select all the data folders and then delete them.

Summary for maintaining the experiment:
Save the Experiment daily to save calibration values and data folders from PCR runs.
Use archival Experiments to collect groups of data folders
Keep the number of data folders in the main Experiment under 30
Transfer History (if desired) to an external Notebook file
Delete History and unneeded data folders after they are saved

Note: Always operate with only one main experiment. The main reason (among many), is that if two were used, the calibration values would not be current on both.

Start-up and shut down

Start-up
1) Turn the main, green switch ON
2) Wait for Windows to completely boot up (10 minutes)
   (if prompted, the user password is "llnl")
3) Open the desired Igor experiment

Shut-down
1) Save the current experiment
2) Press Ctrl+Alt+Delete
3) Respond to prompts (end tasks, shutdown, …)
4) Turn off the main power switch when prompted

Note: Quitting Igor takes about 20 seconds, during which time nothing seems to be happening. This can be done before the Ctrl+Alt+Delete but it isn't necessary.

Section 3. Main menus

The PolyHanaa provides a complete menu bar with standard (File, Edit…) selections for high level operations, built-in (Igor) menus for data handling and LLNL generated menus developed specifically for the 24.

When the active window is a graph (or table), Igor will automatically generate a corresponding 'Graph' (or 'Table') menu. This can be used to make the graph look a particular way.

There are four LLNL generated menus. They are all prefixed by “p.” to distinguish them from the Igor menus. These include ‘p.Setup’, p.‘Calibration’, ‘p.Trends’ and ‘p.Analysis’. Analysis is only available when no runs are in progress.

Setup Menu
The Setup menu is used to set assay types, cycle times, cycle temperatures, integration times and positive ID variables.

Assign Chambers
Assign Chambers is used to tell the system which chambers will be in use for the next PCR run and what assays will be run. The assignments determine what will be displayed on the real-time graphs and which chambers will be tested for positives (any label other than "not used"). The panel for assigning chambers allows the user to select names (IDs) from popup lists and apply them to any subset of chambers.

If many temporary IDs are to be used, it is more efficient to enter the labels directly. This can be done using the table ‘Chamber_Status_Waves’. This is a Table Macro accessible via the menu path Window > Table Macros > Chamber_Status_Waves. It shows several of the key waves associated with the PolyHanaa, including the waves ‘IDa’ and ‘IDb’, which determine the ID names on the front panels. (Refer to Note under next topic (Add to Assay List) regarding entering values in Igor tables.) After the desired IDs are properly entered and the table is closed, use the Macros menu item ‘Reset Panels’ to activate the new labels for the next run.

Add to Assay List
Add to Assay List is used to add assay names to the popup list for future convenience. Names other than those on the popup list can be entered directly in the Chamber_Status_Waves table. This table can be found via Windows > Table Macros.

Note: If you make direct entries into the spreadsheet, be careful to cut and paste regions of exactly the same size. The Igor default is to change the size of the arrays without a warning– it will not tell you that the regions are different. Also, a value is not fully entered if a check mark still appears in the box at the top of the window. You must select the check box or the return key to fully enter the value.

Removing IDs from list;
Open the Assay_List table under Windows/Table Macros
Select the cell name(s) you wish to delete
Select Delete Points... under the Data menu
Select the Do It button

Note: Always be sure the chamber assignment labels are accurate before starting a run. The values are kept in the save files after the run and should accurately reflect the run conditions. Also, if a chamber is not used and the label indicates it is, the software will process the data as if the chamber contained a sample. As a result, a false positive could be found based on noise alone. The real-time plots would also include the noisy data (normalized).

Set PCR Parameters

Set PCR Parameters is used to enter setpoint temperatures and hold times for PCR cycles (and any denaturation or reverse transcriptase steps). There are several methods for loading parameters, depending on user preferences and whether the changes are minor or apply to all groups. The Set PCR Parameters panel interface can be cumbersome in some cases.

Note: The panel changes **all** parameters for the group(s) selected to those that currently appear. This could cause a problem if you intend to change only one of the parameters.

Note: The value for the first step in Section 1 must be non-zero. If you don't want a Section 1 setting, make the temperature equal to the first step of Section 2 and the time = 1 second.

The View All Parameters table shows the current settings for all 6 groups. Values can be entered directly into this table. Again, be careful to copy and paste equal numbers of cells.

The View Template Sets table on the bottom half of the display shows commonly used template profiles that can be entered by the user. A set can be loaded by selecting the appropriate column then using Load Set (below).

In order to change the name of a set, do the following;

Right-click on the set name then select the Rename (second) option.

Enter the desired name.

Close the table with the close box (Not the Close button on the panel) and select the Replace option. (Alternatively, you could select the table, type Cntrl + Y, then select the Update Window Macro checkbox.)

Note: If you don't replace/update the macro after changing a name, the next time the table is opened, an error will appear because the old wave will no longer be defined. In this case, the best solution is to use the 'Rename...' option under the Data menu to rename the newly changed wave back to the name that the error said was missing. After doing that, the table should open and you can try the above procedure again, being sure to replace/update the changes.

Load Set

Load Set will load the selected template set (above) into the Set PCR Parameters panel, at which point the user can then select which groups the values should be applied to. When finished, the Close button will close the panel and both tables.

Set Integration Times

Set Integration Times is used to enter detector integration times. These times should be long enough to get approximately 200 to 500 counts from the detectors from samples before the PCR starts. This can be tested using the utility r4d(time A, time B, chamber), entered on the Command Line or by using the Table Macro ‘All_Diodes’ directly after a run (or partial run). In the Analysis mode, the values appear in the table displayed by the menu item ‘Show Raw Signal Table’. If a reading is greater than 950, it may be saturated, in which case the integration time should be lowered. If the reading is above 950 at the beginning of a run, it will not be able to increase because it is already at the maximum value allowed by hardware.

When using Set Integration Times, the values accepted are limited to certain nominal ranges. Values other than these can be entered directly and they will work, but if they need to be used, something may be wrong with the chemistry. These nominal ranges are as follows:

Channel A: 0.1 seconds to 1.4 seconds
Channel B: 0.1 seconds to 0.8 seconds

Note: The Channel A assay is inherently weaker and may need more time.
Note: If an integration time of 0 is entered, the system will actually integrate for 5 milliseconds. This setting may be useful when using trends, as the trend stops during integration times, resulting in a discontinuity in the liquid waveform.

Note: During integration, the heaters are turned off (to minimize electrical interference). As a result, the temperature will droop somewhat. The droop is negligible for short integration times (less than 1 second), but should be checked if the combined integration time approaches 2 seconds. The droop can be checked using the Trend menu.

View Integration Times
View Integration Times shows a table which can be used to verify detector integration times (or enter them directly).

Set Positive ID Variables
Set Positive ID Variables is used to set system variables associated with the positive calling algorithm. Typically (with the possible exception of the threshold values), these variables are rarely changed. Be sure you understand the implications before you change them.

Normalization cycle for signals: If the value is 10 (typical), then all plots will = 1 at cycle 10.

Cycle to start testing for positives: Software won't start looking for a positive until it reaches the cycle value displayed (typically = 12)

The bottom two fields define the cycle number range displayed on the 10 Signal graphs.

Cycle Range for calling positives used to set the level at which the positive ID algorithm calls a positive. Raising this number requires a steeper slope for a positive to be identified. The macro allows for individual values for each chamber.

Hit threshold corresponds to a change in the normalized signal (or signal ratio) from one cycle to the next. If that level exceeds the threshold level for 3 out of 4 cycles, the system will announce that a positive has been detected. Decisions about what levels are proper are best determined by using the Data Analysis experiment to analyze a PCR run to see how the values correlate to the signal curves.

Notes regarding tuning the positive ID of the system;
There are several adjustments to tune the level of positive calling depending on user needs. If the user is interested in being advised of any potential positive as soon as possible, the system can be configured to do so. In such a case, the user assumes the responsibility for verifying a true positive vs. a false positive. On the other hand, if the user wants to rely exclusively on the algorithm, the system can be configured to trade speed and sensitivity

for the certainty of no false positives (but with the risk of missing some weak positives). Of course, the user can always choose to call a signal positive even though the system did not.

Key parameters:
- positive ID threshold level - increase in normalized signal for a data point to count as a 'hit'. Typical level = 0.01; Conservative level = 0.03
- Hit pattern - number and frequency of hits required before positive is called. Typical criterion (built-in) = 3 out of 4 cycles; Conservative = 4 out of 5. To change the setting requires changing the variables 'gPosCount' to 4 and 'gPosRange' to 5.
- Minimum voltage level - no positives unless voltage exceeds limit. Typical level = 0.1 volts; Conservative level = 0.3 volts

The Set Positive ID Variables panel also includes a checkbox for flattening the baseline on signal graphs. If the checkbox is checked, the baseline will automatically be flattened.

Clear Analysis Panels

Clear Analysis panels is the same as 'Return to Run Mode' and would only be used if there was a problem in which, after using the Analysis mode, the analysis panels remained active and the analysis menu (and hence 'Return to Run Mode') was inactive.

Calibration Menu

The calibration procedure should take approximately 5 minutes per chamber. It determines heater values (0-1023) corresponding to sample temperatures of 94, 57 and approximately 75 degrees Centigrade.

Be sure that the calibration insert is full (25 microliters) before calibrating. The actual level isn't critical for standard (non-TAG) calibrations, but the calibration won't be reliable if the insert becomes empty. It is best to add 5 microliters of mineral oil on top of 20 microliters of water to reduce evaporation.

Calibrate

Calibrate opens a panel with which the user can select a chamber to be calibrated and whether or not to use the graph option. More than one chamber can be calibrated at a time, but only one chamber per group at a time. Occasionally, errors may occur when all 6 groups are being calibrated at once. If a particular chamber fails to calibrate in such a case, it should work the next time. The calibration result layout will indicate conclusively if the calibration was successful (see below).

Note: The first stage of the autocalibration is to establish a baseline response to the ambient temperature level. To do this, the fan is turned on to bring the sample to room temperature. The time to do this is currently set for 12 seconds, which should be sufficient in most cases. However, user should be aware that 12 seconds may not be long enough if the calibration sample was not near room temperature when the run was started. In such a case, the calibration graph would show a changing baseline at the beginning of the calibration and the calibration result could be in error.

Note: When calibrating an entirely new module, enter initial values into the Calibration Table as follows: 470 (Pt94), 280 (Pt57), and 375 (Pt7_).

Note: If system won't let you start a calibration, wait for any calibrations in progress to finish, then enter 'initCalSys()' on the command line.

Note: If you get a history message saying "TC already in …" when starting to calibrate, it may just be that the touch pad interpreted a click as a double click and tried to perform the same operation twice. This message is normally given to keep the user from trying to calibrate two chambers in the same group at once. A chamber can only be calibrated with the thermocouple (TC) positioned in front of that group.

Show Calibration Graph
Show Calibration Graph opens the most recent calibration graph for a particular group

Show Calibration Result
Show Calibration Result opens an Igor 'layout' which contains a summary of the calibration results. The layout can be printed for documentation purposes. Some of the key calibration information is also displayed in the History window.

Stop Group 1 Cal – Stop Group 6 Cal
The 'Stop Group # Cal' series stops the calibration procedure for a given group. This menu would be used to stop a calibration if the graph (with its Stop button) wasn't being displayed.

Show Module Numbers
Show Module Numbers opens a table which shows which chamber modules serial numbers are in which slot (1-24). Since there is no way for the system to know this, this table must be kept current by the user. It is only used for documentation purposes.

Show Calibration Table
Show Calibration Table opens a table which shows the current calibration values for all chambers. **Warning:** These numbers should never be altered by the user as they directly control the temperature the chambers reach. The table is provided for documentation, so the user can see what the values were when the table was printed. Note that the table entries beyond 24 contain the previous values that were determined by the previous calibration. These values determine the change in the current calibration. Note: If the offset value changes, the other values are likely to change substantially. If the offset value doesn't change, then, as a rule of thumb, for every 5 units the calibration values change, the sample temperature will change 1 degree Centigrade.

Toggle A Graph
If the graph option was selected for a calibration series and more than one of the first three ('A') groups (1, 2 and 3) are being calibrated at the same time, the 'A' graph will only show one of the three. In this case, the Toggle A Graph menu item will toggle to the next of the three. The hot key shortcut is useful for this is; 'Alt + C' then 'Alt + A', where the first pair activates the calibration menu and the second pair activates Toggle A Graph.

Toggle B Graph
This is the same as above, except for the B groups of 4, 5 and 6.

Trends menu

All the trend menu items require a thermocouple to be inserted into a given chamber. The connector of the thermocouple must be connected to the corresponding group's socket (with the exception of 'TC as External Probe'). Stopping any trend will also stop all active chambers.

Note: With trends, data stops during normal integration times, resulting in a discontinuity in the liquid waveform. If you are monitoring hold temperatures, integration times can be set to 0 (which the system interprets as 5 milliseconds), and the discontinuities will not be noticeable. If you want to see the temperature drop during integration (the heater is off during integration times), use the 'TC as External Probe' option (below).

Monitor a Profile

Monitor a Profile will run the selected chamber with whatever profile is currently loaded. This trend allows you to see if the desired temperatures and hold times are being achieved.

TAG Adjust 5s_5s

TAG Adjust 5s_5s is used to adjust TAG points. The selected chamber is run with the current temperatures but the hold times are temporarily set to 5 seconds to make the process faster.

Note: TAG values are tuned up automatically (if they are already close) when a chamber is calibrated when fast TAGs are in use (ref. Macros section below)

Temperature Adjust

Temperature Adjust allows the user to adjust the calibration values directly to have the sample temperatures match the current profile. This could be considered to be an alternate way of calibrating. Be sure to let the chamber profile stabilize by not adjusting anything for at least 2 or 3 cycles. The hold times for this trend are whatever is currently entered in the system unless TAG points are used, in which case the times are 5 seconds. Therefore, you can tell if the hold times need to be changed if the temperature is not at the desired temperature for the desired time.

Caution! Be sure you know what temperature the profile is set for when you make adjustments. Otherwise, you could introduce a calibration offset. (While you will be adjusting the calibration values for 94 and 57, the actual values sent to the heater are extrapolated values corresponding to the temperature currently in the PCR parameters profile for the particular group.)

TC as External Probe

TC as External Probe allows the user to use a single thermocouple to move from chamber to chamber across all groups to determine which chambers may need calibration. In this case, the groups to be tested need to be started manually. If you plan to test the group the thermocouple is from, start that group before starting the trend. If not, the trend will stop when the run starts and you'll have to start the trend again. It is OK to start other groups after the trend has started. When the trend is stopped by the button, all running groups will be stopped automatically.

Note: During the trend, the groups' panels will not update cycle number.

Show Trend Graph

Show Trend Graph shows the latest trend graph data graph. This graph will contain the platinum trace – even if the most recent trend was an external TC trend (which doesn't show the platinum trace because it is confusing (especially with autoscaling) due to the fact that it doesn't change much).

Analysis menu

Overview
The analysis menu is used when no PCR runs are active. It is used to review or analyze data from recent PCR runs using Igor's data folders. Each data folder contains data from a particular run, where a run can contain anywhere from one to 24 chambers, depending on how it was initiated. Start All and Start Some (in the main panel) will typically contain several groups together as a designated run. When a group is started using the group's Run button, only that group will be in the run.

Select Data Folder
Select Data Folder prompts the user to select from the existing data folders. If the user wants to see data from a data folder that has been moved to an archive experiment, the Data Browser can be used (see discussion regarding 'Browse Expt…' in the data folders section above) to move a copy back to the main experiment.

After selecting a data folder, Continue will present a panel and graph for each of the groups used in the run. The graphs titles contain the run number.

Note: If want to quit from this panel, DON'T use the Quit button. Rather, select a data folder and then use the Return to Run Mode menu option.

Test New Threshold
Test New Threshold allows the user to change threshold parameters (or any parameter) and see how the positive calling algorithm responds with the new conditions. Note (as indicated on the panel) that leaving 0's as new threshold values results in no change. If several groups were on the run and the threshold is only changed on one group, all the other groups will get recalculated with no change in the process.

Note: There is no need to worry about overwriting threshold values that were used on the actual run. The original values are preserved and are used whenever the data folder is selected using Select Data Folder.

The Analyze Group series
The 'Analyze Group #' series opens a table which shows all the arrays associated with the positive calling algorithm, including the signal level from the graphs. Note: If you try to analyze a group not on the run, a (harmless) "unknown keyword" error will result. A similar error about a "missing wave" will occur if analysis hasn't recently taken place (in which case the threshold values may be ambiguous). In such a case, verify the threshold values and select Test New Threshold before analyzing a group.

Clear Overlay Folder
Clear Overlay Folder is used when overlay plots are being generated. Overlay plots can have waves from different runs overlaid on one graph for comparison. An overlay folder

gets loaded with the assay waves of interest, one at a time. Before starting this process, use Clear Overlay Folder to start with a clean slate.

Add to Overlay Folder
Add to Overlay Folder lets user select the next wave to add to the overlay data folder. If there are no more waves in the data folder selected, <none> will appear as an option. In this case, select 'No' (Continue will give an error).

Show Overlay
Show Overlay plots all the waves in the overlay data folder on the same graph. The Graph menu can then be used to change how the various curves are displayed. Double clicking on a given trace will automatically bring up the modification dialog.

Leave the overlay mode (overlay folder is now the active folder) by using the 'Select Data Folder' or 'Return to Run Mode' menu selections.

Show CT Table
Show CT Table opens a table which shows the CT (threshold cycle) for each chamber used. Note that the threshold cycle is different from the cycle at which the algorithm calls a positive (PCA & PCB). Typically, the difference is about 3 cycles. For the PolyHanaa, the threshold cycle corresponds to the cycle number where the slope of the signal waveform first crosses the (user entered) threshold slope value. Note that this is different than the standard value of where the waveform leaves the baseline.

Show Pos ID Setup Table
Show Pos ID Setup Table opens a table which shows the values used during the run

Show Raw Signal Table
Show Raw Signal Table opens a table which shows the raw diode signal level (a number between 0 and 1023) for all the chambers used on the run.

Run Report for DF
Run Report for DF builds a table of the run results for all run data folders within the currently selected data folder. The table contains run number, chamber, assay label and whether it was positive (PRESENT) or negative (not detected). If an embedded folder (containing other run data folders) is within the current data folder, it will be ignored. If you want a run report for a group of runs that are currently stored in several embedded data folders, all the run data folders must first be copied to a single data folder.

The desired data folder can be selected in two ways. The easiest way is probably to drag the red arrow in the Data Browser to the appropriate folder. Having the Data Browser open, with all the embedded folders apparent, makes the run report much easier to define. The second method is to use the Select Data Folder menu item. If you go one level below the desired data folder (i. e., one of the run data folders) and then select Quit Macro, the red arrow will be at the desired data folder. This can be verified by watching what happens in the Data Browser as you use Select Data Folder.

Return to Run Mode
Return to Run Mode quits the analysis mode and returns the user to the normal run mode. If any graphs or tables are not automatically closed in the switch, close them in the run mode. (They will appear between the seams of the panel/graph mosaic.)

The Macros menu

Start BkGrd task
This starts the BackGround task. Normally this happens automatically. It should only be used if the background stops due to a system error, in which case the software gets fooled. That is, the software still thinks the background task is on when it is really off. Starting it with the menu item sets everything right again.

Note: If the system stops the background task, the following message will always appear in the History window: “Background stopped due to expression error”. Whenever this happens, the system will not operate correctly until the background task is restarted.

Stop BkGrd task
This stops the BackGround task. Normally this happens automatically, so this menu item will virtually never get used.

Reset Command Window
This resets the Command Window to fit in its normal place in the mosaic.

Reset Panels
This resets the group panels to their standard positions. This is useful if some were moved intentionally or if one was moved inadvertently.

Reset Graphs
This resets the group graphs to their standard positions. This is useful if some were moved intentionally or if one was moved inadvertently.

Set TAGs to 1
This sets the TAG (Touch And Go) points to 1.0. When they are 1, they have no effect and the system can be considered to be running without TAG points.

Use Fast TAGs
Use Fast TAGs sets the TAG values to whatever they have been determined to be previously. If only select chambers have valid TAG points, the other chambers will retain TAG values of 1 and operate normally.

Note: In a given group, all used chambers must be either 1 or TAG values.

The TAG values can be viewed in the Calibration Table under ‘chambers’ 25 through 48. Whatever values are in the table for HiFF and LoFF for chambers 1 to 24 are the current TAG values.

The Data Browser

The Data Browser (shown above) is found under the 'Data' menu. It shows all the data folders within the experiment. During normal run mode, the root folder is active. The red arrow indicates which is the active folder. When a data folder is selected from a popup menu in Analysis, that folder becomes the active folder (and gets the red arrow). The red arrow can be dragged to another data folder manually as well, although this can fool the

software, resulting in having some menu options become inactive. Basically, if the root is active, all menus except Analysis will be totally active and if the active folder is not the root, only the Analysis menu (of the p.* menus) will be active.

Contiguous groups of data folders can be deleted by 'lasooing' them (click then drag) slowly from the right hand side towards the left.

The Data Browser is a very versatile tool. There is a good help section associated with it that shows all the features.

Section 4. Operational Notes

Warnings: … be advised!

Use only one experiment for all PCR runs. That is, **never open an old experiment and run PCR** with it. The reason is that the calibration settings for an old experiment will probably not match current hardware conditions. Also, any system settings or preferences may not be up to date.

When system says "**File already open**" after you try to save experiment, select OK then repeat the save command. It always seems to work the second time. This is annoying , but if you proceed as if it was saved, the information may be lost. (Actually, it can probably be recovered by opening the most current 'T' file on the desktop.)

Spin down samples before running to avoid air bubbles interfering with the detection.

When **copying and pasting** in tables, the paste region must be the same size as the copy region. If it isn't, Igor will assume you want to change the wave (array) dimensions and will expand/contract the wave without alerting the user.

Be aware of '**Background stopped due to expression error'** printed in the history area. This can confuse the LLNL software and keep the system from operating properly. Refer : Macros menu [Start BkGrd task].

Anything in the history window with a "******" prefix is an alert of a potential problem. Pay special attention to these when diagnosing any problems.

Helpful Tips & Info

Startup takes a long time (be patient, this is normal)

Be careful using the **touchpad** – it may respond to a gentle, unintentional tap when you don't want it to. In some cases, it could also interpret a single tap as a double tap and stop an action as soon as it was started.

Use the **Hot Key** shortcuts (standard underlined menu items for PC) to speed up operation and minimize use of touch pad.

Igor is **case insensitive**, so the command line doesn't care about CASE.

Never run with Data Browser open because it slows down system operation.

Minimize moving cursor during operation. This takes computation time from the processor and may result in lost data or communications glitches.

Delete excess **'T' work files** from desktop. A new one is generated each time the system alerts that the "File is already open" when trying to save.

Check **memory status** via double click on My Computer or under Proc A with windows explorer utility.

The system automatically checks for **broken chambers** upon startup. This feature can be used if you suspect a broken chamber.

If chamber modules from Group 6 need to be removed, the flat panel **display cable** must be unplugged. It is OK to plug it in and out with the system On.

May **lose data for initial cycles** if Section 1 hold time is < 15 seconds and all 6 groups are started at once.

If you **inadvertently Start** a group, you have to wait until the history says that group is running before it will accept a Stop button.

It's OK (but not good) if a group sticks at the end of a run and never posts the final cycle or the red "F". Use the stop button if it seems to be stuck. The panel may update at that point if the Macro 'Reset Panels' is used.

Since signal waves are normalized to values at **cycle 10**, they don't exist prior to cycle 10. This explains why the graphs don't exist until then.

The **posted cycle number** (and time) corresponds to the last completed cycle.

Assays labeled as positive controls will appear **pink** if positive to distinguish them from a sample that is positive (which appears as bright red).

CT values are printed to the history window during PCR runs and analysis.

When interpreting platinum values (0-1023) used in **calibration setpoints**, the rule of thumb is that a change of 5 units corresponds to a change of one degree centigrade.

Specific Operational Guides

<u>Guide for using TAGs and setting hold times</u>
Touch and Go (TAG) points are heater setpoints that precede the actual hold setpoints. They are multipliers to the hold setpoints, thus, the high TAG point is higher than the high hold setpoint by a (typical) factor of 1.2 and the low TAG point is lower than the low hold setpoint (typically by a factor of 0.88). Note that if the TAG points are set to exactly 1, they have no effect.

TAG points are used to significantly shorten cycle times by making the transition times much less. When the heater goes to a setpoint, the hold time starts when the heater gets there, not the liquid. Since the heater is very fast and the setpoint is a steady state value that corresponds to the appropriate temperature, the liquid will take a while to get to the steady state after the heater is holding. Because of this, without TAGs, an entered hold time of 10 seconds may result in the liquid never reaching the setpoint.

In general, you should add 10 to 15 seconds to the desired hold time to have the liquid see the desired hold time.

With TAG points, the heater setpoint is set beyond the hold setpoint. The key is that the heater changes to the hold value as soon as the heater reaches the TAG point. In other words, the TAG setpoint has a zero hold time (hence the name Touch And Go). The bottom line is that, when the TAG points are properly set, the liquid temperature will have very sharp transitions (or 'knees') because the setpoint will drop right when the liquid has reached the desired temperature.

While using TAG points can shorten cycle times, using them is somewhat problematic:

- The settings may not be as stable as the hold settings since they are dynamic vs. steady state quantities.
- It takes more time to maintain / verify proper TAG points (although the calibration routine does some tuning).
- Care must be taken not to set a high TAG point too high or the sample could boil.
- If a TAG point is accidentally set too low, the fans may not be able to cool that far (i.e., if beyond ambient).
- They introduce a risk of chambers overheating. If the system controller accidentally sticks at a TAG point, a very high steady state temperature will result. The risk of controller failure may be higher if several chambers in the same group are used.

The bottom line is you can use TAG points, but start slowly to assess the risks. Using one chamber per group is probably the safest approach. (It's OK to have one chamber in a group with fast TAG points and the other chambers with TAG points = 1.) See also: Macros Menu [Use Fast TAGs]

Guide for determining proper integration times
Refer to: Setup Menu [Set Integration Times]

Guide for understanding the positive calling algorithm
Refer to: Setup Menu [Set Positive ID Variables]

Guide for navigating to the desktop
Accessing files on the desktop is a bit convoluted in Windows NT.

The path is c: > winnt > profiles > administrator > desktop

Guide for using desktop backup files

After any run that has proceeded past 10 cycles has ended (stopped or finished), in addition to saving data to a data folder within the Experiment, an archive/backup file set of data is also sent to the desktop. These files may never be needed. If they are needed as a backup, they can be activated by dragging and dropping them onto the Igor Pro application icon. This icon can be located by using the standard 'Windows NT Explorer' utility that is available when the Windows logo key is pressed on the keyboard. The path is c: > program files > wavemetrics > igor pro folder > igor.

Guide for interpreting Status Table waves

The table 'Chamber_Status_Waves' is a Table Macro accessible via the menu path Window > Table Macros > Chamber_Status_Waves. It shows several of the key waves associated with the status of the group panels, assay results and operation of the polyHanaa. The waves displayed in the Status Table are as follows;

Group indicates which group each chamber belongs to and which position it has in that group.

statusA and statusB refer to the status of the colored assay boxes on the front panels:
1 = Positive (red)
2 = Negative (green)
3 = Running (yellow)
4 = Not Used (gray)
5 = Positive Control that is positive (pink)

IDa and IDb are the assay assignments for the A and B assays, respectively. This is where the user can change ID assignments directly (rather than using the Assign Chambers popup panel).

PCA and PCB are the Positive Cycle values for the A and B assay. The positive cycle is the cycle after which the positive calling algorithm determined the sample to be positive.

Done is a wave that tracks whether each group is done (1) or cycling (0). This wave controls much of the system operation so it serves as a valuable diagnostic if something goes wrong with normal operation.

CTa and CTb are the Threshold Cycle values calculated from the signal values when a sample is called positive. The CT values are typically about 3 cycles less than the PC values above due to the way the algorithm works (refer to: Setup Menu [Set Positive ID Variables])

Guide for setting positive ID variables

Refer to: Setup Menu [Set Positive ID Variables]

Section 5. Troubleshooting Guide

Also refer to: Operational Notes [Warnings / be advised!]

System-wide problems

If the Igor opens the experiment in the 'minimized' mode
Windows will look compressed. Click the full screen icon and reopen experiment.

If password information is requested
Windows has been set to log in automatically. If this feature fails, the proper keys are:
user name = Administrator
password = phanaa1!

If get a message about 'can't find another Igor user'
Have to Stop/Start the entire machine. Some data may be lost.

If system seems to be running slowly or unreliably
- The experiment may be too large (>2 Mb). See Maintaining the Experiment.
- The background task stopped due a system error. Refer to: Macros menu [Start BkGrd task].

If the Experiment becomes corrupted
If the Experiment appears to be corrupted, save it as a special name, then re-open the most recent backup experiment. (Note: you may want to save the History and any new Data Folders before closing.)

Problems with thermal cycling:

A chamber doesn't calibrate
- Chamber is chipped or cracked (auto-tested upon system startup)
- Try it again doing nothing different (occasional glitches do happen)

A chamber gives bad temperatures after a calibration
- Chamber is chipped or cracked (auto-tested upon system startup)
- Experiment was not saved after most recent system calibration

Chamber(s) not heating or heating slowly
- Chamber is cracked or broken (auto-tested upon system startup)
- Entire group will stop if one chamber is dead (fan never resets/stops)
- Section 1 settings are set to 0. They must be non-zero for the unit to cycle.

Excessive overshoot in thermal profile
- TAG points are being used with unoptimized values

Problems with signals, results:

Diode readings have excessive noise
- Raw signal level is very low (less than 50)
- Chamber has no sample insert
- LED is not coming on (should see green/blue flash after fan)
- Liquid level in insert is very low (2 mm below neck)

Signal graph is flat or becomes flat during run

- Detector saturated (raw signal greater than 950)

Positive controls are giving negative results
- Chamber is not reaching temperatures
- Chamber is cracked or broken (auto-tested upon system startup)
- LED is not coming on (should see green/blue flash after fan)
- Positive ID threshold is too high (graph would look OK)

Positive identification is inconsistent with signal curves
- ID threshold levels are too high or low
- Diode signals are very weak.

Some diode data is missing
- This will happen rarely. Software patches the graph as B24(i) = B24(i-1)
- Initial cycles lost if Section 1 is < 15 s and Start All at once.

Problems with modules or inserts:

Module won't fully insert into slot
- The removable filter holders may not be completely inserted

Insert won't pull out of chamber module
- The chamber may have overheated and melted the insert internally. Refer to: Recovery Procedures.

Loss of liquid in inserts after run
- Liquid has condensed in the cover
- Insert is leaky

Problems using software:

If you inadvertently overwrite a Graph, Table or Panel Macro, you should Save (the current version) As... (something else) and then activate a **copy** of the backup version for the next run.

If PCR menus **and** Analysis menus are grayed out
- Type and enter 'gNoAnalysis = 1' on the command line
- Refer to section on the Data Browser for additional information

A window doesn't close or seem to respond correctly
- The window has a _1 suffix. [ref. Recovery Procedures below]

A window gets corrupted with a 'ghost' button attached to it
- Use the Reset Panels or Reset Graphs (Macros) menu option
- Notify LLNL if persistent

Problems entering table values
- The typed in value was not entered (by clicking check box or using return key)
- Cell groups of different sizes were copied or pasted

Section 6. Recovery Procedures

Note: A chamber module can be checked off-line for this open circuit condition by measuring the resistance across the top two pins on the 5-pin connector. The resistance should measure around 100 Ohms. If it measures infinite (open), the internal contacts are not making proper contact.

If you assigned "not used" to a used chamber;
Data is still collected if a chamber (in a running group) is assigned the label 'not used', but the signal wave and positive ID analysis is not done. The data that is automatically saved after the experiment will also retain the raw data. The user can therefore regenerate the desired curves and information after the fact in either the Run mode or in Analysis mode.

Two waves must be modified for each assay. If it was a B assay that was improperly assigned 'not used', the waves are 'statusB' and 'IDb'. For an A assay they would be 'statusA' and 'IDa'. The statusA/B needs to be changed from 4 to 3. The IDa/b needs to be changed from 'not used'.

Warning: Don't try to use Assign Chambers to do this – it will cause more problems.

In Run mode, before starting a new run on the group (and when there are no other groups running), open the table macro Chamber_Status_Waves (refer to: Operational Notes [Guide for interpreting Status Table waves]). Adjust the table entries as mentioned above for the specific chamber number involved (you shouldn't be changing anything other than '4' to '3' and 'not used' to the appropriate ID). The signal curves can then be regenerated for a given group by entering the special function AfterTheFact(gn) on the command line, where gn is the appropriate group number (from 1 to 6).

Note that this will not update the Analysis data folder as the original data was already saved. However, since the Analysis mode regenerates the data each time the data folder is called, corrections can be made to the waves in the appropriate data folder such that it will subsequently analyze the run as if the assay label was correct. Corrections can be made as follows:

In Analysis mode, once you are in the appropriate data folder, enter the following on the command line (or select it here and press Ctrl + enter);

```
edit statusA,statusB,IDa,IDb
```

Warning: You can't use the Chamber_Status_Waves table (or any Table Macro for that matter) in the Analysis mode because the waves shown will be from the root data folder, not the analysis data folder.

You can then edit the table waves appropriately by changing the appropriate 'not used' entries for the A or B assays and by changing the status value for the same assay(s) from '4' to '3'. To check the result, close the table then use the menu item 'Rerun Analysis', being sure the proper group is selected. Note that Rerun Analysis doesn't require threshold changes to run – as indicated on the panel, leaving 0s in the panel fields is interpreted as no change. Selecting 'Continue' should display the desired information.

If a window doesn't respond as expected;
If a window (table, graph, panel) doesn't seem to respond or to close, it is probably because Igor tried to open a window that was already open. When it does so, it creates a window that looks like the original but is named with the suffix '_1 '. Such a window will not respond properly. This can be verified by clicking on the window then typing the key combination 'Command + Y'. The **name** (not the title) of the window will be shown. If the window can't be closed with the close box, it can be closed by directly by executing a 'DoWindow/k' command in the Command Window. For example, if the window name is LLNLpanel_1, then type 'DoWindow/k LLNLpanel_1' on the Command Line and press return.

If a chamber overheats and insert melts;
The polypropylene inserts become soft if the chamber overheat and will rupture inside the chamber module. If they are pulled out when the chamber is beginning to overheat, they may pull out or they may stretch like taffy or only part may pull out. Once the chamber module cools down, the insert will probably be stuck, having conformed to the windows in the heater walls.

While the sample will most likely be lost, the chamber may be recoverable. Call LLNL for assistance. The chamber is made of two halves, so it may be possible to extract the insert once the module is disassembled. If so, if the heater doesn't fracture in the process, it may be operational again. The first test would be a recalibration.

If a chamber's fan stays On of Off;
You can confirm the problem by listening and by comparing trend cooling/ heating rates. To recover, type 'resetFan(#)' on the command line, where # = the chamber number (1 though 24).

If system comes up with chambers still cycling (after a system crash);
Type 'stopSMCs()' on the command line, where # = the chamber number (1 though 24).

Section 7. Maintenance Functions

These functions are helpful when testing system performance prior to PCR runs or when troubleshooting a problem. The names are short because they are typically typed onto the command line and executed.

rad(ta,tb); Read All Diodes (integration time A, integration time B)
This will attempt to read all the system diodes with the entered integration time as the temporary value. A table will contain the results. If the table is incomplete, fill in with r4d (below);
Note: Due to a hardware error, chambers 23 and 24 may not respond.
Note: If an integration time of 0 is entered, the system will use 5 ms.

r4d(ta,tb,cn); Read Four Diodes (time A, time B, chamber)
Four diodes are read and printed to history (and to Read_All_Diodes table) when a given chamber number (cn) is entered; the A and B channel for the cn entered and the A and B for its (SMC) mate.

TC(gn); ThermoCouple (group number)
Prints (to history) the temperature for the group specified or for all 6 groups if enter TC(0).

sd(gn); Show Diodes (group number)
Shows table of raw signal level (0-1023) for any assays being used in group gn.